proteins in yeast or bacteria, facilitating basic research and enabling the more efficient production of therapeutic proteins.

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TTBK2 Kinase: Linking Primary Cilia and Cerebellar Ataxias

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Mutations disrupting primary cilia cause retinal, renal, and cerebellar defects, and misregulated Sonic hedgehog signaling. A new mouse mutant in the TTBK2 kinase fails to make cilia, and shows neural tube and Sonic hedgehog signaling defects. Ciliary targeting mutations in human TTBK2 are linked to spinocerebellar ataxia, suggesting cilia protect from neurodegeneration.

Primary cilia are highly conserved signaling organelles assembled with a ciliary membrane extended over the centriole-nucleated axoneme with nine microtubule doublets. These tiny hair-like structures are exquisitely positioned within tissues to receive neuroendocrine and sensory signals, notably via G-protein-coupled receptors and olfactory receptors. In vertebrates, cilia are especially important in regenerating tissues, where cell-cycle and specific morphogen pathways, including the Sonic hedgehog pathway (Shh), facilitate developmental patterning and regeneration of ciliated tissue. In Shh responsive cells including neural progenitors, Shh signaling also requires cilia to process pathway components (Goetz and Anderson, 2010). Specific tissues, including the retina and kidney, show distinctive degeneration in genetic diseases that have defects in ciliary function, called ciliopathies. Among neural structures, the neural tube, cerebellum, and corpus callosum appear particularly reliant on coupling cilia to the Sonic hedgehog pathway for neural morphogenesis and maintenance. In this issue of *Cell,* the Anderson lab describes *bartleby*, a new embryonic lethal mouse mutant with strong neural tube and Shh signaling defects. These defects derive from a mutation in TTBK2, a protein kinase important for transitioning centrioles from the cell cycle to postmitotic assembly of axonemes (Goetz et al., 2012). TTBK2 is also an allele of the human SCA11 spinocerebellar ataxia (Houlden et al., 2007), providing a link between cilia and neurodegeneration. Like the obstinate scribe from Melville's classic story, *bartleby* prefers not to make any cilia!

The Anderson lab has been the leader in establishing the cilia-hedgehog link; they used the alkylating agent ENU to induce mutagenesis in the mouse and screen for phenotypes typical of ciliary loss: holoprosencephaly, alterations in body axis and limb formation, randomized heart looping, and mid-gestation lethality (Huangfu et al., 2003). The *bartleby (bby)* mouse showed this characteristic phenotype. Dorsal-ventral patterning markers were defective with a loss of ventral neural tube markers and structures, and Shh markers were lost in the limb bud. Fulfilling their expectations, examination of cilia formation by the marker Arl13b showed no cilia in the neural tube or in *Ttbk2*^{bby} mouse embryo fibroblasts.

Ultrastructure examination showed *bby* embryos presented fully intact mother centrioles, which were appropriately docked to the ciliary membrane, as seen by the CEP164 marker of the membrane proximal distal appendage. The ninein marker of the subdistal appendage, important for microtubule nucleation, was also intact. However, although the basal body was properly assembled, the axoneme, which is normally assembled

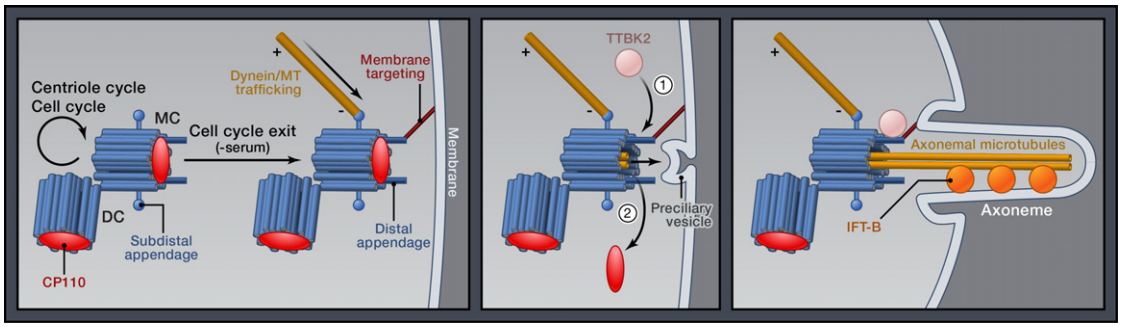

Figure 1. Centriole Duplication Occurs during the Mitotic Cell Cycle

Serum withdrawal induces cell-cycle exit and the recruitment of the centriole to the apical surface of cells via the distal appendage and dynein-based microtubule traffic to the subdistal appendage. The TTBK2 kinase is recruited to the centriole and transition zone near the ciliary base, and causes the loss of CP110, an inhibitor of axonemal growth, thereby licensing growth of the axoneme, assembly of the preciliary vesicle ciliary, and recruitment of intraflagellar transport proteins, including IFT-B. IFT-B plays an additional role in axonemal and ciliary membrane growth and in the maintenance of the mature cilium.

from the basal body, was fully absent. Markers for the transition zone, the ciliary gate apical to the centriole were intact. Thus, there appeared to be a block in the steps between recruiting the centriole to the membrane and building the axoneme.

Steps in ciliary assembly typically begin with the mother centriole docking to the plasma membrane (Figure 1), organized by the distal appendage. The subdistal appendage can then recruit dynein-dependent microtubule-based trafficking to the now apical centriole to provision the growing or mature cilium with essential components. To construct the actual cilium, trafficking of a vesicles from the recycling endosome population (Westlake et al., 2011) allows Rab-dependent vesicle fusion and the assembly of a large preciliary vesicle between centriole and membrane (Sorokin, 1962). This vesicle flattens and bends to form a preciliary sheath, which fuses with the plasma membrane to cause the cilium to erupt apically. The axoneme assembles within the forming sheath. At this early point, components of the intraflagellar transport (IFT) complexes help assemble the cilium. IFT components are also important in dynamic trafficking of receptors and signaling components within mature cilia.

In cultured, nontransformed cells, ciliation is triggered by serum withdrawal, causing cells to withdraw from the cell cycle. The centriole has its own duplication cycle, which allows production of two centrioles to form the poles of the mitotic spindle. During the elongation of the centriole, the protein CP110 assembles at the tip of the growing centriole but is capable of blocking the ability of centrioles to nucleate axonemes. This restrains the daughter centriole from making axonemes and requires that the mother centriole lose CP110 during the transition to axonemal growth. In cultured cells, serum withdrawal triggers the loss of CP110 (Spektor et al., 2007). The persistence of CP110 at both centrioles can block axoneme formation, and indeed overexpression of CP110 blocks ciliation altogether. In *bby* mutants, CP110 is present on both centrioles. These mutants are unable to remove CP110 from mother and trigger axoneme growth. The authors also find that intraflagellar transport proteins are not recruited to centrioles in *bby* mutants, suggesting that the loss of CP110 initiates both axoneme assembly and the linked recruitment of IFT proteins.

Mapping and sequencing revealed that the mutation was a premature termination of TTBK2, the tau tubulin kinase 2, a Casein Kinase 1 family member. TTBK2 can phosphorylate the microtubule nucleating protein tau and bind to microtubules. Although the biochemical properties of this kinase are poorly understood, previous screens had linked TTBK2 to Shh and to cilia, and the direct binding to microtubules suggested that TTBK2 could play some role in axoneme function (Goetz et al., 2012).

The authors find that TTBK2 reversibly localizes to the basal body and transition zone in response to serum control, putting the protein at the right place at the right time to regulate CP110. Moreover, TTBK2 kinase activity is required for ciliation and the 936 amino acid C-terminal domain is required for centriolar localization and rescue of ciliogenesis in *Ttbk*^{bby} mouse embryo fibroblasts. Thus, key biochemical functions of TTBK2 are required for ciliogenesis. Future work will establish whether TTBK2 directly phosphorylates CP110 or whether the mechanism is more elaborate.

CP110 was characterized in a network of centriolar proteins including CEP97 that is anchored to the centriole by CEP290/NPHP6 (Spektor et al., 2007). The gene encoding CEP290 is mutated in patients with retinal degeneration and Joubert syndrome, a neurological disease with cerebellar aplasia. The cerebellum develops from the embryonic hindbrain and first rhombomere lip by a complex morphogenetic process that requires both cilia and Sonic hedgehog signaling. Thus proteins in the network regulating CP110 are linked to cerebellar development.

The TTBK2-CP110 connection is rendered more provocative by the link of TTBK2 to a mutation in spinocerebellar ataxia, called SCA11 (Houlden et al., 2007). Ataxias (from the Greek for "lack of order") result from a group of genetic syndromes with progressive degeneration of the cerebellum and spinal cord, causing uncoordinated movements in gait, eye movement, speech, and other motor coordination skills. The spinocerebellar ataxias (SCAs) are the most common and result from diverse mutations in over 30 genes (Matilla-Dueñas, 2012). Some SCA genes are called

ataxins (ATXN). A recent connection between cilia and cerebellar ataxia was suggested because CEP290 copurified with the proteins NPHP5, another ciliopathy protein, and ataxin10, which itself was homozygously mutated in a patient with Joubert syndrome (Sang et al., 2011). Further supporting the connection, the authors find that SCA11-associated variants of TTBK2 do not rescue cilia formation in *Ttbk2*^{bby} fibroblasts and interfere with ciliogenesis in wild-type cells in a dominant negative fashion. Provocatively, several SCAs show additional phenotypes characteristic of ciliopathies, including retinal degeneration. It may be productive to examine whether other ataxias are linked to cilia.

Cilia are present in almost all regions of the brain, frequently near ventricles where they may receive hormonal signals from the soma or elsewhere in the brain. Only a few specific signaling molecules, notably GPCRs like the somatostatin receptor SSTR3, have been found in cilia (Berbari et al., 2008). Although failures in specific endocrine receptors would be expected to lead to subtle endocrine or sensory defects, the absence of some receptors may create sensitivity to excitotoxic neural cell death, causing progressive loss of neural tissue in adult life. Adult defects can be subtle or inconsistent, making consistent syndromic presentation hard to define. Cerebellar defects, linked to ataxias, are the most identifiable defects linked to cilia, but a much broader group of neurodegenerative disorders linked to ciliary loss may be hiding in the complexity of human variation and limited diagnostics. Indeed, cilia may protect from a number of neurodegenerative diseases. It may be a good time for neurologists to dig deeper into cilia and the molecular pathways explaining ciliopathies to find explanations for other neurodegenerative diseases.

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Capturing the Cloud: UAP56 in Nuage Assembly and Function

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The nuage is a hazy electron-dense structure unique to germ cells and is enriched in components of the piRNA pathway. Although the nuage is cytoplasmic, Zhang et al. now show that it is organized by an intranuclear protein, UAP56.

The nuage (from the French word meaning cloud) is a hallmark of the germline cytoplasm in diverse organisms. Recent studies have revealed the steps of nuage assembly and its role in posttranscriptional silencing of transposons via the Piwi pathway (Voronina et al., 2011). In *Drosophila*, nuage assembly is hierarchical with Vasa, a DEAD box RNA-dependent helicase required for germline development and piRNA biogenesis, at the apex (Figure 1). Vasa is required for nuage localization of Tudor and Tudor-domain-containing (Tdrd) proteins such as Partner of Piwi (Papi), which then recruit Piwi proteins Aubergine (Aub) and Ago3 by binding to their symmetrically dimethylated arginine residues (Harris and Macdonald, 2001; Liu et al., 2011; Malone et al., 2009; Nishida et al., 2009). Thus, Tudor and Tdrd proteins might serve as a platform for the assembly of piRNA biogenesis pathway components, including those for "ping-pong amplification." To this mix of cytoplasmic players, Zhang et al. (2012) now reveal an unexpected role for a nuclear protein, UAP56, in nuage assembly.